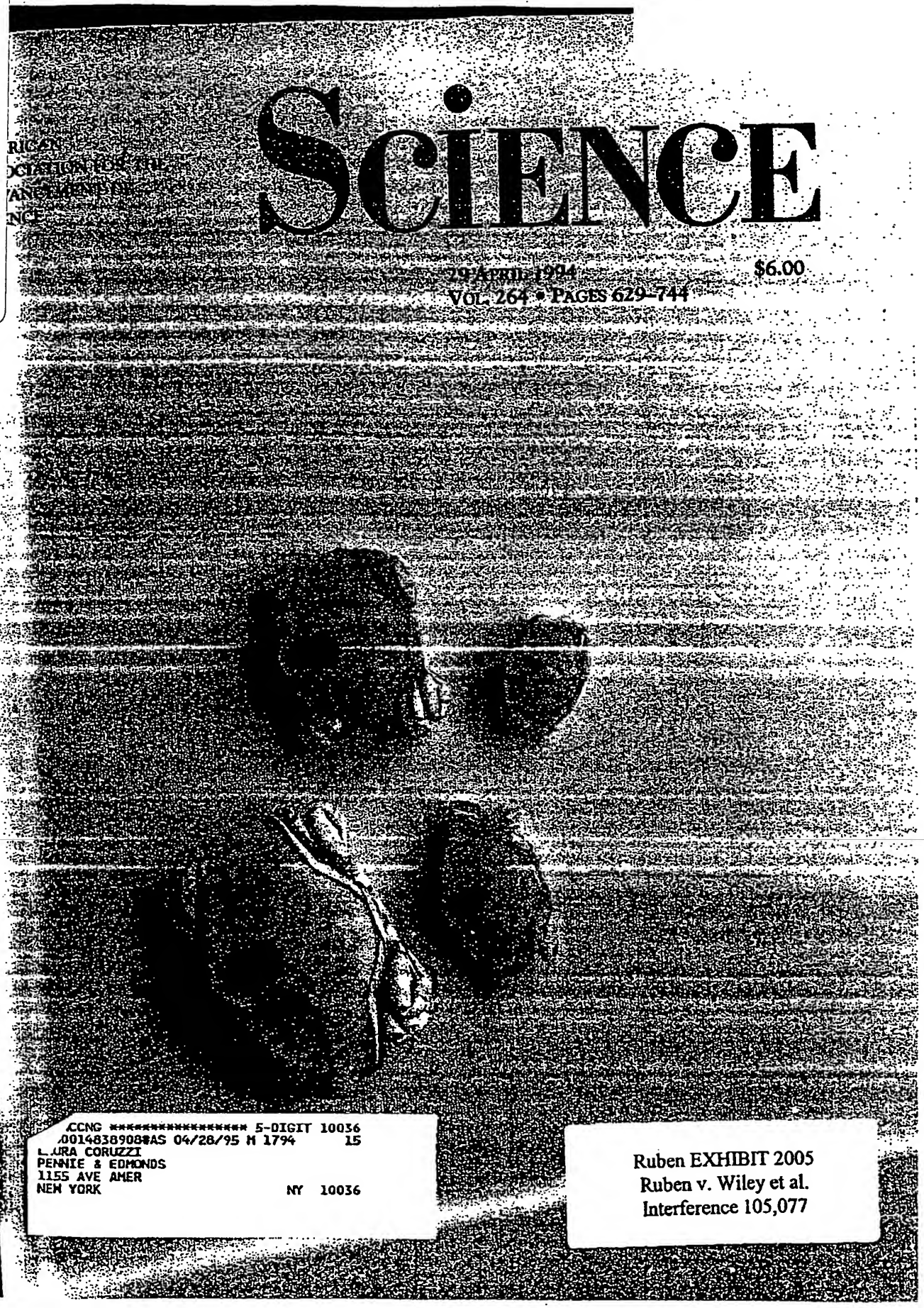


PERSPECTIVES

Unraveling Function in the TNF Ligand and Receptor Families

Bruce Beutler and Christophe van Huffel

Tumor necrosis factor (TNF) is a mixed blessing for higher organisms: It can protect animals against infection, yet is also known to induce shock and inflammatory disease. Its close homolog, lymphotoxin (LT), elicits a similar spectrum of biological actions. Both molecules are homotrimers and both engage the same two plasma membrane receptors.

With last year's discovery that LT subunits may exist in a heteromeric form with subunits of yet another member of the ligand family (dubbed LT-β), and with the observation that the heteromers of LT-α and LT-β could not engage the two known TNF receptors, an orphan ligand came into being (*1*). What is the function of this heteromer? And what are the broader functions of LT-α implied by the existence of the heteromer?

In a report in this issue of *Science*, Crowe and co-workers (*2*) show that LT-α–LT-β, the cell-bound cytokine heteromer, engages the so-called TNF receptor–related protein (TNFRrp). In a related report in this issue, De Togni and colleagues (*3*) have deleted the mouse LT-α gene and have observed a startling phenotype—a complete lack of lymph nodes—which immediately suggests an essential function for the TNFRrp/LT-α–LT-β receptor-ligand couple in lymph node development.

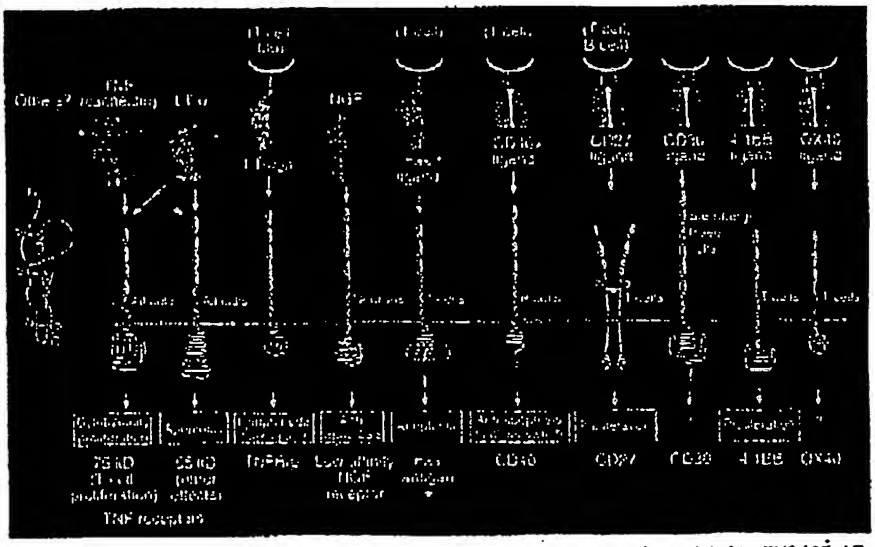
Each member of the TNF receptor family (there are 10, excluding viral and fungal homologs) has a characteristic repeating extracellular cysteine-rich motif (*4*, *5*). The family of ligands for these receptors has nine representatives (see figure). Two of the ligands (the TNF and LT-α homotrimers) are each capable of binding to two of the receptors (the 55- and 75-kD TNF receptors). All of the other ligands and their receptors interact, as far as is known, with one-to-one correspondence (each ligand has one, and only one, receptor target). All of the TNF ligand family members are believed to be trimeric proteins, and all exert their effects by causing receptor multimerization at the cell surface (*6*–*8*). The LT-α homotrimer is entirely secreted, the TNF homotrimer is mostly secreted, and the other family members are predominantly type II transmembrane proteins.

What cellular processes do these receptor-ligand pairs mediate? Although the cytoplasmic domains of most members of the receptor family are remarkably divergent from one another, some of the TNF receptor family members clearly generate cytotoxic signals when activated, whereas others promote growth. The 55-kD TNF receptor and the Fas antigen transduce cytotoxic signals. The 75-kD TNF receptor may also exert a cytotoxic effect, although likely through a different mechanism (*9*). It also has, however, a clear ability to stimulate thymocyte proliferation (*10*).

Considerable insight into the essential functions of several members of the TNF receptor family has already been gained from the identification or creation of mutations that abolish the expression of the individual proteins. Naturally occurring mutations of the Fas antigen and its ligand cause lymphoproliferative disease (*11*–*13*), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T cell–dependent B cell activation (*14*–*17*). Targeted mutations of the low-affinity nerve growth factor (NGF) receptor cause a disorder characterized by faulty sensory innervation of peripheral structures (*18*).

In the past year the genes for the two TNF receptors have been deleted in mice (*19*–*21*), and genes encoding inhibitor proteins that selectively bind and neutralize homotrimeric forms of TNF and LT-α have been introduced into mice (*22*–*23*). Deletion of the 55-kD TNF receptor gene causes pronounced immunodeficiency, in which animals show heightened susceptibility to *Listeria monocytogenes*. "Knockout" of the 55-kD receptor is also associated with resistance to the lethal effect of lipopolysaccharide in galactosamine-treated animals. Deletion of the 75-kD receptor gene causes a minimal phenotype, in which scab formation fails to occur in response to repeated intradermal injection of TNF, and there is modest resistance to the lethal effect of TNF (*21*).

Combination of the 75- and 55-kD receptor knockouts has not yet been reported. However, functional ablation of the homotrimeric forms of TNF and LT-α has been accomplished by inhibitor gene transduction (*22*). Such animals presumably lack any input through either of the two known TNF receptors, and indeed present



The TNF ligand and receptor families. Ligands are shown as crystallographic models [the TNF (*27*), LT-α (*28*), homotrimers, and NGF (*29*)] or as models based on tertiary and quaternary structure of the TNF homotrimers (the LT-β subunit and Fas ligand). Blue atoms on the surface of the TNF, LT-α, and LT-β trimers have been implicated in receptor binding. The basic form of the repeating, cysteine-rich unit (blue) is shown at left (*2*). The tertiary structural characteristics of the cytoplasmic domains are unknown; red box indicates region of homology shared by the 55-kD TNF receptor, the Fas antigen, and the CD40 molecule. Asterisks: proteins with natural mutations that cause disease.

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an excellent phenocopy of both of the individual knockout strains, showing pronounced lipopolysaccharide resistance, TNF resistance, defective scab formation, and *Listeria* sensitivity. Once more, however, no developmental functions were revealed by any of these experiments.

Therefore, the results of De Togni and co-workers showing a developmental defect in the LT-α knockout mouse are at first surprising. Deletion of the LT-α gene results in a distinctive phenotype, characterized by the absence of lymph nodes and by disordered splenic architecture. Because the two "classical" (55 and 75 kD) TNF receptors cannot be implicated as essential participants in the ontogeny of lymph nodes and spleen, either by gene knockout or by inhibitor gene transduction studies, suspicion immediately centers on the heteromeric ligand (LT-α–LT-β) and its receptor, TNFRrp.

TNFRrp was, before the new findings of Crowe and co-workers, known only as a complementary DNA clone (*24*). Recognized as a member of the TNF receptor family on the basis of homology, it was a receptor in search of a ligand. Now known to be an essential part of the ligand for TNFRrp, LT-β was originally identified as a "tether" for LT-α subunits. This finding explained why a proportion of LT-α is in a cell-bound state—an observation that originally could not easily be reconciled with the fact that the protein, as initially translated, lacks the transmembrane domain that characterizes all other members of the ligand family (*25*).

It now seems likely that TNFRrp serves an essential organogenetic function. But what is the nature of this function? Because mice lacking the LT-α–LT-β heteromer have normal numbers of peripheral blood monocytes, T cells, and B cells, mutation of TNFRrp does not forbid the differentiation of these cells or their survival. Rather, it prohibits their organization to form lymph nodes.

Lymph nodes are structures of mesodermal origin. Lymphocytes are recirculated through the lymph nodes by way of endolymphatic recirculation, a process that depends on homing receptors located on the high venular endothelium of vessels supplying lymph nodes and Peyer's patches (*26*). It is tempting to consider that the LT-α–LT-β heteromer and its receptor may somehow be involved in homing, but much more work remains to establish this. Further, next to nothing is known of the forces that initiate the aggregation of lymphocytes, macrophages, and other cells that form lymph nodes.

The studies of De Togni and co-workers at once underscore the limitations and the power of gene knockout procedures as an analytical technique, particularly when applied in the study of multigene families. Deletion of a single gene may affect the function of more than one protein. As such, the deletion of the LT-α gene has led to the ablation of both the LT-α homotrimer and the LT-α–LT-β heteromer. In contrast, single gene deletion may be insufficient to reveal the function of a protein, given the existence of functionally redundant molecules. Thus, LT-α gene knockout cannot be expected to fully ablate signal transduction through the 55- and 75-kD receptors, which can be triggered by the TNF homotrimer as well as the LT-α homotrimer. One prediction offered by the results of Crowe and De Togni is that deletion of the LT-β gene or the TNFRrp gene, or the use of inhibitor molecules capable of engaging the LT-α–LT-β heteromer would also prevent the formation of lymph nodes.

Is the developmental function of the LT-α–LT-β heteromer and its receptor mediated by the triggering of a signal transduction pathway in the receptor-containing cell, upon a simple mechanical interaction, or upon a combination of the two? Mutational analysis of the TNFRrp cytoplasmic domain might well provide an answer.

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Cell Death Genes: *Drosophila* Enters the Field

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Large numbers of apparently healthy cells die during the lifetime of an animal (*1*). These normal cell deaths have a distinctive morphology, called apoptosis: The cell and its nucleus condense and often fragment, and the cell or fragments are rapidly phagocytosed either by neighboring cells or macrophages (*2*). The deaths are thought to be suicides in which the cell activates an intrinsic death program and kills itself; for this reason, the process is often called programmed cell death (PCD) (*3*). PCD is regulated by cell-cell interactions so that the organism can eliminate unwanted cells (*4*). Remarkably, although the death program is a fundamental property of animal cells, it is still not known how the cells die. A significant step toward solving this puzzle is reported in this week's issue of *Science*, in which Steller and his colleagues describe the first gene in the fruit fly *Drosophila* that seems to be specifically involved in PCD (*5*).

The strongest evidence for an intrinsic death program in animal cells originally came from genetic studies in the nematode *Caenorhabditis elegans*. Two genes, *ced-3* and *ced-4*, were shown to be required for the 131 normal cell deaths that occur during the rapid development of the mature hermaphrodite worm, which contains only about 1000 somatic cells (*3*). Both genes must act in the dying cells or their close ancestors, and if either gene is inactivated by mutation, none of the 131 cell deaths occurs. *ced-4* encodes a novel protein (*6*), whereas *ced-3* encodes a protein that is homologous to the mammalian cysteine protease interleukin-1β (IL-1β) converting enzyme (ICE) (*7*), which in macrophages cleaves the IL-1β peptide from a larger pre-

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